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ISOLATION OF ANTISENSE OLIGONUCLEOTIDES

Technical field

The present invention relates to a method of isolating antisense oligonucleotides from other components of a biological solution.

Background

Biotechnological methods are used to an increasing extent in the production of proteins, peptides, nucleic acids and other biological compounds, for research purposes as well as in order to prepare novel kinds of drugs. Due to its versatility and sensitivity to the compounds, chromatography is often the preferred purification method in this context. The term chromatography embraces a family of closely related separation methods, which are all based on the principle that two mutually immiscible phases are brought into contact. More specifically, the target compound is introduced into a mobile phase, which is contacted with a stationary phase. The target compound will then undergo a series of interactions between the stationary and mobile phases as it is being carried through the system by the mobile phase. The interactions exploit differences in the physical or chemical properties of the components in the sample.

Interactions between a target compound and metal chelating groups present on the stationary phase are utilised in a chromatographic purification method denoted immobilised metal ion adsorption chromatography (IMAC), also known as metal chelating affinity chromatography (MCAC), which is often used for the purification of proteins. The principle behind IMAC lies in the fact that many transition metal ions can coordinate to phosphate groups and nitrogen atoms, such as in the amino acids histidine, cysteine, and tryptophan, via electron donor groups on the amino acid side chains. To utilise this interaction for chromatographic purposes, the metal ion must be immobilised onto an insoluble support. This can be done by attaching a chelating group to the chromatographic matrix. Most importantly, to be useful, the metal of choice must have

a higher affinity for the matrix than for the compounds to be purified. Examples of suitable coordinating ions are Cu(II), Zn(II), Ni(II), Ca(II), Co(II), Mg(II), Fe(III), Al(III), Ga(III), Sc(III) etc. Various chelating groups are known for use in IMAC, such as iminodiacetic acid (IDA), which is a simple chelator, and nitrilotriacetic acid (NTA), which is a tetradentate chelator. Elution of an IMAC resin is conventionally performed by lowering the pH.

In recent years, IMAC has successfully been used for the purification of proteins and peptides, wherein His-tags have been introduced by recombinant techniques to facilitate efficient purification thereof by IMAC. For this reason, IMAC has assumed a more important role in large-scale protein and/or peptide production. In addition, IMAC has also been used in purification of phosphorylated proteins and peptides from tryptic protein digests. Such phosphorylated proteins and peptides can subsequently be analysed by ESI/MS/MS to determine the phosphorylated sites therein.

Further, during the period when the IMAC was relatively new, use thereof for purification of various compounds were suggested. For example, Porath et al (USP 4,677,027) disclosed in 1985 how biological macromolecules and particles can be separated using a product consisting of a solid phase having immobilised metal ions on its surface substituted via a metal chelate bond with a polymer. The envisaged biomolecules are virus and cells, but polysaccharides, proteins and also oligonucleotides are mentioned. However, since then, oligonucleotides have due to more recent scientific findings found new applications, in turn necessitating novel modifications thereof.

One example of a more recently developed field, wherein oligonucleotides are modified, is the antisense technology in drug discovery. Antisense drugs work at the genetic level to interrupt the process by which disease-causing proteins are produced. This is possible, since proteins have been shown to play a central role in virtually every aspect of human metabolism. Almost all human diseases

are the result of inappropriate protein production, or a disordered protein performance. This is true of both host diseases, such as cancer, and infectious diseases, such as AIDS. Traditional drugs are designed to interact throughout the body with protein molecules that support or cause diseases. Antisense drugs are designed to inhibit the production of disease-causing proteins. They can be designed to treat a wide range of diseases including infectious, inflammatory and cardiovascular diseases and cancer and have the potential to be more selective, and, as a result, more effective and less toxic than traditional drugs. The mechanisms behind antisense technology have been widely described, see e.g. Uhlmann et al in *Antisense Oligonucleotides: A New Therapeutic Principle*, Chemical Reviews, Vol. 90, Number 4, June 1990. In brief, as is well known, during transcription of DNA into RNA, the two complementary strands of the DNA partly uncoil, whereby the strand known as the sense strand separates from the strand known as the antisense strand. The antisense strand is then used as a template for transcribing enzymes that assemble mRNA in the process known as transcription. The mRNA then migrates into the cell, where ribosomes read the encoded information and string together amino acids to form a specific protein in the process known as translation. Now, the antisense drugs are complementary strands of small segments of mRNA, and they can be either DNA or RNA. To create antisense drugs, nucleotides are linked together in short chains known as oligonucleotides. Each antisense drug is designed to bind a specific sequence of nucleotides in its mRNA target to inhibit production of protein encoded by the target mRNA.

The linking together of oligonucleotides can be performed in any kind of commercially available automated solid-phase synthesiser for synthesis of oligonucleotides under cGMP conditions for clinical studies and commercial drug supplies.) In such synthesis, the oligonucleotides, wherein one oxygen atom of the phosphate group of each base in the native nucleic acid has been exchanged for a sulphur atom, are easily produced. However, an inherent problem in the synthesis of such thioated oligonucleotides, herein-denoted antisense oligonucleo-

tides, is the fact that it will be practically impossible to perform with a yield of 100% correctly phosphorothioated oligonucleotides. Instead, a yield in the range of about 70-75% is usually obtained. Accordingly, before any antisense drug can be prepared thereof, the synthesised product will require a subsequent purification in order ensure a sufficient quality.

Reverse phase HPLC is commonly used for purification of antisense oligonucleotides. However, use of high pressures is in general not considered to be advantageous conditions for this kind of process, since it put high demands on the equipment used and also makes the process difficult, and consequently costly, to scale-up.

Deshmukh et al (Deshmukh, R.R., Miller, J. E., De Leon, P., Leitch, W.E., Cole, D.L., and Sanghvi, Y. S. in "Process Development for Purification of Therapeutic Antisense Oligonucleotides by Anion-Exchange Chromatography", Organic Process Research & Development 2000, 4, 205-213) describes the development of an anion-exchange chromatography method for purification of phosphorothioate antisense oligonucleotides. More specifically, 20-mers which are antisense inhibitors of the cell adhesion molecule ICAM-1 were synthesised and subsequently purified on an anion exchanger carrying quaternary ammonium functional groups on a polystyrene-based matrix (Source 15 and Source Q 30, both from Amersham Biosciences AB, Uppsala, Sweden). The most advantageous resolution is observed for the higher pH value tested for elution, which was pH 11. However, it has still to be shown whether or not a fully thioated 20-mer can be separated from a 20-mer, wherein one or more of the target oxygens have not been substituted with sulphurs. Thus, the selectivity obtainable with ion exchange for purification of antisense oligonucleotides is still not fully satisfactory. In addition, another disadvantage is that such purification of antisense oligonucleotides by anion-exchange chromatography will also require a step of desalting afterwards, which involves a further process step and consequently a higher process cost in total.

Similarly, Deshmukh et al (Deshmukh, R.R., Warner, T.N., Hutchison, F., Murphy, M., Leitch, W.E., De Leon, P., Srivatsa, G. S., Cole, D.L., and Sanghvi, Y. S. in "Large-scale purification of antisense oligonucleotides by high-performance membrane adsorber chromatography", Journal of Chromatography A, 890 (2000) 179-192) have suggested purification of antisense oligonucleotides using strong anion exchange membranes. However, like in the above described method, the selectivity obtainable is still not fully satisfactory (is this true, can we add any other disadvantages /differences). In addition, use of membranes entails a low capacity and hence large size membranes will be required for a reasonably efficient process. Finally, this method will like the above-discussed anion-exchange also require a step of desalting afterwards.

Thus, in summary, there is still a need of alternative procedures for the purification of antisense oligonucleotides.

Summary of the present invention

One object of the present invention is to provide a method of isolating antisense oligonucleotides from corresponding sense oligonucleotides in a biological solution. This can be achieved by the method as defined in the claims. A specific object of the invention is to provide a method of isolating antisense oligonucleotides from a biological solution, which method exhibits an improved selectivity as compared to the prior art methods.

Another object of the invention is to provide a method of isolating antisense oligonucleotides from a biological solution, which method reduces the need of organic solvents and/or high pressures as compared to prior art methods.

A further object of the present invention is to provide a method of isolating antisense oligonucleotides from a biological solution, which method is easy to scale up and hence more cost-effective than the prior art methods.

Other objects and advantages of the present invention will appear from the detailed disclosure that follows.

Brief description of the drawings

Figure 1 shows an example of a seven-base full-length, fully thioated phosphorothioate (a); its monophosphodiester analogue (b) and a single deletion sequence (c).

Figure 2 shows IMAC using Fe^{3+} as metal ion as described in Example 1 below and illustrates a comparison of elution of two different oligonucleotides with the same sequence of bases.

Figure 3 shows IMAC using Zr^{2+} as metal ion as described in Example 2 below and illustrates a comparison of elution of two different oligonucleotides with the same sequence of bases.

Definitions

In this specification, the term "oligonucleotide" is used in its conventional meaning, i.e. to mean a sequence of nucleotides, and the term "polynucleotide" refers to a longer sequence of nucleotides than the oligonucleotide.

The term a "nucleotide" means a residue comprised of three parts, namely an inorganic phosphate, a simple sugar and either a purine or a pyrimidine base. In each nucleotide, the three parts are attached to each other in the order -phosphate - sugar - base -. In an oligonucleotide, ester bonds link the sugar and phosphate components of adjacent nucleotide monomers. Since the sugar and the phosphate within a nucleotide monomer are also linked via an ester bond, the sugar- phosphate- sugar linkage along the backbone of a poly- or oligonucleotide chain is known as a phosphodiester bond.

The term "chromatography" encompasses chromatographic separation methods performed in packed columns, in expanded or suspended beds and on membranes.

The term "resin" refers to the solid phase used in chromatography, i.e. the adsorbent that captures the target species. A "resin" may be produced in the form of porous or non-porous spherical or essentially spherical particles, beads, such as beads for expanded bed adsorption, and monoliths. Further, by providing the

resin on a support, membranes can be provided, which are also useful for isolation of a species from a liquid. A resin is also known in this field as a matrix. The term "adsorption" means herein the binding of a species to a ligand on a resin.

The term "eluent" is used herein in its conventional meaning i.e. for a solution capable of perturbing the interaction between the solid phase (resin) and product (target species) and promoting selective dissociation of the product from the solid phase.

Consequently, the term "desorption" means to perturb the interaction as explained above.

The term "buffer" or "buffered solution" refers to a mixture of acid and base which when present in a solution reduces or modulates changes in pH that would otherwise occur in the solution when acid or based is added.

Detailed description of the invention

A first aspect of the present invention is a method of isolating fully thioated single stranded antisense oligonucleotides from a biological solution, comprising the steps of contacting the biological solution with an immobilised metal ion adsorption chromatography (IMAC) resin to adsorb the antisense oligonucleotides to said resin and subsequently contacting the resin with an eluent under conditions that provide desorption of the antisense oligonucleotides from said resin.

Thus, the present invention utilises for the first time to our knowledge the interaction of a metal with the backbone phosphothioate group of a nucleic acid in the purification of antisense oligonucleotides. Without wishing to limit the present invention to any specific interactions, it is also assumed that the nitrogen atoms of one or more of the bases adenine, guanine, uracil, cytosine and thymine of the oligonucleotide may also be involved in this binding.

In the present context, it is to be understood that the term "fully" thioated means that in 100% of the phosphate backbone groups present in a corresponding native oligonucleotide, one of the non-bridging oxygen atoms in the phosphate backbone has been replaced by a sulphur atom.

The IMAC resin used in the present method can be any resin, such as the once exemplified in the section "Background". In brief, metal chelating groups include for example the iminodiacetic (IDA) group, the tris(carboxymethyl)-ethylenediamine (TED) group, the N-(hydroxyethyl) ethylenediaminetriacetic group, and derivatives such as the N-(methyl), and the N-(hydroxymethyl) IDA groups. These groups can be cross-linked to the natural or synthetic polymeric support by standard aliphatic ether linkages and reagents, such as bisoxirane, epichlorhydrin, and 1,4-bis-(2,3-epoxypropoxy)butane. Examples of natural polymeric support materials are e.g. agarose, alginate, carrageenan, gelatin etc. Synthetic polymers can be illustrated by styrene or derivatives, divinylbenzene, acrylamide, acrylate esters, methacrylate esters, vinyl esters, vinyl amides etc, optionally cross-linked with any conventional cross-linker, such as divinylbenzene, di- or polyfunctional (meth)acrylate esters, di- or polyfunctional (meth)acrylamides, triallylisocyanurate, divinylamides. For clarity, in this context, it is understood that an IMAC resin as used in the present method is comprised of a support to which chelating groups have been attached, and charged with coordinating ions. Examples of suitable coordinating metal ions are e.g. Al, Ce, Cu, Co, Fe, In, Ga, Ge, Lu, Ni, Ru, Sb, Sc, Sn, Yc, Zn, Zr, Ta and Th ions. IMAC resins are also commercially available, such as HiTrap™ Chelating HP Columns and Chelating Sepharose™ Fast Flow, both from Amersham Biosciences AB, Uppsala, Sweden.

In this context, it is understood that the term "resin" is used to encompass particles and beads as well as monoliths and membranes.

The desired antisense oligonucleotides can be separated from many kinds of components of the biological solution, such as proteins or sense oligonucleotides, in large depending on the nature of the biological solution. Thus, in one embodiment, the biological solution is provided from an automated synthesis of antisense oligonucleotides. Hence, in this embodiment, the biological solution is a synthesis solution, and in a similar embodiment, the biological solution is a solution wherein the antisense oligonucleotides have been synthesised using non-automated methods. Thus, synthesis can be performed in solution according to well-known methods or in any commercially available kind of equipment, such as a ÄKTA™ oligopilot (Amersham Biosciences AB, Uppsala, Sweden). In another embodiment, the biological solution is serum, such as human serum, and the purpose of the method can then be to quantify the antisense oligonucleotides present therein. This embodiment can be part of treatment scheme, wherein it is desired to test the presence of drug i.e. antisense oligonucleotide in the blood of the patient.

In one embodiment, the isolated single stranded (ss) antisense oligonucleotides are of a size in the range of 10-30 bases, such as 15-25 bases and more specifically 18-21 bases. In a specific embodiment, the antisense oligonucleotides are of a size in the range of about 18-20 bases. In another embodiment, the antisense oligonucleotides are comprised of up to about 25 bases, such as up to 20 bases. In yet another embodiment, the antisense oligonucleotides are comprised of at least 5 bases, such as at least about 10 bases. However, in this context, since it is well-known that the kind of condition to be treated using the antisense technology will decide the nature, such as the base sequence and the size, of the antisense oligonucleotide, it is understood that the present invention also encompasses shorter or longer oligonucleotides as well, if they are useful in an antisense technology-based drug. Such drugs are useful in the treatment of both host diseases, such as cancer, and infectious diseases, as discussed in further detail in the section "Background" above.

However, as also indicated in the background section above, the synthesis of antisense oligonucleotides often results in part in incorrectly synthesised antisense oligonucleotides. The most common impurities in a biological solution that results from such synthesis are deletion sequences, i.e. antisense oligonucleotides which are one or more bases shorter than the desired product. Such deleted oligonucleotides can be described as (n-1) mers, (n-2) mers, (n-3) mers etc, wherein n denotes the number of nucleotides of the desired full-length product. Thus, in one embodiment of the present method, the fully thioated antisense oligonucleotides are separated from incorrectly synthesised oligonucleotides. Other examples of incorrectly synthesised oligonucleotides are addition sequences, i.e. antisense oligonucleotides that are longer than the desired products, and branched products.

Another example of undesired components in a biological solution resulting from antisense oligonucleotide synthesis is incorrectly thioated sequence, i.e. not fully thioated oligonucleotides. Thus, in one embodiment of the present method, fully thioated antisense oligonucleotides are separated from incorrectly thioated antisense oligonucleotides. The most common examples of not fully thioated oligonucleotides are the ones wherein one, two or three phosphodiester groups have not been correctly thioated. Further examples of incorrectly thioated oligonucleotides are for example 20-meric oligonucleotides wherein one phosphodiester group has not been correctly thioated, and hence oligonucleotide which are about 95% thioated are separated from the fully thioated ones. Similarly, a 19-meric, 18-meric or 17-meric oligonucleotide wherein one base has not been correctly thioated is thioated to about 94%. Accordingly, in one embodiment, the present fully thioated antisense oligonucleotides are isolated from oligonucleotides that are thioated to about 90%, such as about 94%, and preferably to about 95%. In another embodiment, the present fully thioated antisense oligonucleotides are isolated from oligonucleotides that are thioated to about 40%, preferably to about 60%, more preferably to about 80% and most preferably to about 90%.

In the prior art, when proteins and/or peptides have been isolated using IMAC, conditions of neutral or close to neutral pH, such as about 7.5-8.0, have been utilised. The present inventors unexpectedly found that when antisense oligonucleotides are isolated using IMAC, a lower pH is more favourable. Thus, in one embodiment of the present method, the conditions for adsorption are defined by a pH value below neutral. In a specific embodiment, the pH is adapted to below about 7, such as below about 5, and even below about 3, and more preferably at a pH of about 2.5, i.e. 0-5, 0-3 and 0-2.5, respectively. The pH is easily adjusted by the skilled person in this field by adding a suitable buffer or acid, such as dilute acetic acid. For example, in order to provide a pH of about 2.5, 0.1% acetic acid can be used. As is easily realised, since oligonucleotides are sensitive to extreme pH values, care should be taken not to adjust the pH in any way that can harm the antisense oligonucleotides.

The elution of the desired antisense oligonucleotides from the resin can be performed according to standard methods using an increasing pH and/or phosphate gradient, for example using potassium phosphate. An illustrative gradient is as used in the experimental part below, namely starting from the pH used for adsorption, such as from 0.1% acetic acid to 0.5M potassium phosphate. In an alternative embodiment, the gradient is from 0.1% acetic acid, i.e. about pH 2.5, to 0.2 M potassium phosphate.

Other well-known salts and buffers are also useful for the elution, and the skilled person can easily set the appropriate conditions for elution. As the skilled person in this field will realise, the addition of salt will increase the ionic strength, and hence the pH surrounding the antisense oligonucleotides will change slightly. However, the pH in general during the adsorption of the antisense oligonucleotides will still be lower than the conditions known for use of IMAC for protein separation.

In a specific embodiment, the present method in addition comprises a subsequent step of polishing the isolated antisense oligonucleotides. Such polishing

is easily performed by the skilled person in this field, such as by gel filtration, detritylation precipitation, desalting, change of buffer etc.

Even though the examples shown below utilises a small lab scale, it is understood that the skilled person in this field can easily scale up the present method to a size useful in a production plant. Thus, one advantage with the present method is that it requires less expensive solvents and equipment than e.g. the previously suggested reverse phase chromatography (RPC) method.

A second aspect of the present invention is an antisense oligonucleotide isolated by a method as defined above. Thus, the fully thioated single stranded antisense oligonucleotides according to the invention are obtained in a purity of at least about 80%, more preferably at least about 90% and most preferably at least about 95%, such as close to 100%.

A third aspect of the present invention is the use of an immobilised metal affinity chromatography (IMAC) resin for isolation of antisense oligonucleotides from corresponding sense oligonucleotides in a biological solution. The IMAC resin can be as discussed in relation to the method according to the invention, and the considerations discussed above may also apply to the present use.

Detailed description of the drawings

Figure 1 shows an example of a seven-base full-length, fully thioated phosphorothioate (a); its monophosphodiester analogue (b) and a single deletion sequence resulting in a (n-1) mer (c).

Figure 2 shows IMAC using Fe^{3+} as metal ion as described in Example 1 below and illustrates a comparison of elution of two different oligonucleotides with the same sequence of bases. The X-axis shows the retention volume in ml, while the Y-axis shows the UV absorbance at 260 nm in mAU. One of the oligonucleotides is fully thioated (denoted 20S in Figure 2), while the other one is unmodified (denoted 20P in Figure 2). It appears clearly that the antisense oli-

gonucleotide can be separated from the phosphodiester (non-modified) form of oligonucleotides, the thioated form being eluted as a relatively narrow peak at 7.3 ml, before the unmodified form. The two small peaks eluted early in the chromatogram are presumably synthesis-related, and are caused by impurities in the sample that do not contain any phosphotioates or phosphodiester groups.. Figure 3 shows IMAC using Zr^{2+} as metal ion as described in Example 2 below and illustrates a comparison of elution of two different oligonucleotides with the same sequence of bases. X- and Y-axis are as described above. One of the oligonucleotides is fully thioated (denoted 20S in Figure 3), while the other one is unmodified (denoted 20P in Figure 3). It appears clearly that the antisense oligonucleotide can be separated from the phosphodiester (non-modified) form of oligonucleotides, the thioated form again being eluted as a relatively narrow peak at about 9.4 ml, before the unmodified form. The two small peaks eluted early in the chromatogram are explained as above for Figure 2. A comparison of Figure 2 and Figure 3 reveals a stronger affinity of the oligonucleotides for the Zr-ion than the Fe-ion, however it is noted that the conditions used have not been optimised.

EXPERIMENTAL PART

The present examples are provided for illustrative purposes only and should not be construed as limiting the scope of the present invention as defined by the appended claims. All references given below and elsewhere in the present specification are hereby included herein by reference.

Example 1: Purification of single stranded antisense oligonucleotides by IMAC using Fe^{3+} as metal ion

The oligonucleotides used in this study were 20-mers with the sequence GCC CAA GCT GGC ATC CGT CA. Two different oligonucleotide were used, one fully thioated and one without any modification (phosphodiester form).

For the study was used a small IMAC column with IDA chemistry, the HiTrap Chelating HP column (1 ml volume) (available from Amersham Biosciences AB, Uppsala, Sweden, Prod # 17-0408-01).

The solvents/buffer used in this example are for IMAC rather unusual. As binding "buffer" a solution of 0.1% acetic acid in water was used. The elution was achieved with a 10 Column Volume linear gradient from 0.1% acetic acid in water to 0.05 M potassium phosphate. However, it is noted that these conditions were not optimised, neither for binding (adsorption) nor for elution.

Flow rate of the eluent was 1 ml/min and detection was made with UV at 260 nm.

Thus, the Fe^{3+} was tested and found useful as a metal ion in the method according to the invention. The results of this example are as shown in Figure 2.

Example 2: Purification of single stranded antisense oligonucleotides by IMAC using Zr^{2+} as metal ion

The oligonucleotides used in this study were the 20-mers described in Example 1 above.

For the study was used a small IMAC column with IDA chemistry, the HiTrap Chelating HP column (1 ml volume) (available from Amersham Biosciences AB, Uppsala, Sweden, Prod # 17-0408-01).

In this example, the binding "buffer" was like in Example 1 a solution of 0.1% acetic acid in water. The elution was achieved herein with a 10 Column Volume linear gradient from 0.1% acetic acid in water to 0.2 M potassium phosphate. However, it is noted that these conditions were not optimised either.

Flow rate of the eluent was 1 ml/min and detection was made with UV at 260 nm.

Thus, Zr^{2+} was tested and found useful as a metal ion in the method according to the invention. The results of this example are shown in Figure 3.

CLAIMS

1. A method of isolating fully thioated single stranded antisense oligonucleotides from a biological solution, which method comprises the steps of contacting the biological solution with an immobilised metal ion adsorption chromatography (IMAC) resin to adsorb the antisense oligonucleotides to said resin and subsequently contacting the resin with an eluent under conditions that provide desorption of the antisense oligonucleotides from said resin.
2. A method according to claim 1, wherein the biological solution results from a synthesis of antisense oligonucleotides.
3. A method according to claim 1 or 2, wherein the antisense oligonucleotides are of a size in the range of 5-30, and preferably 15-25, base pairs.
4. A method according to any one of the preceding claims, wherein fully thioated antisense oligonucleotides are separated from incorrectly synthesised oligonucleotides.
5. A method according to any one of the preceding claims, wherein fully thioated antisense oligonucleotides are separated from incorrectly thioated antisense oligonucleotides.
6. A method according to any one of the preceding claims, wherein the adsorption is performed in about 0.1% acetic acid.
7. A method according to any one of the preceding claims, wherein the pH of the biological solution is below about 7 during the adsorption of antisense oligonucleotides.
8. A method according to any one of the preceding claims, which in addition comprises a subsequent step of polishing the isolated antisense oligonucleotides.
9. An antisense oligonucleotide isolated by a method according to any one of claims 1-8.
10. Use of an immobilised metal ion adsorption chromatography (IMAC) resin for isolation of fully thioated single stranded antisense oligonucleotides from a biological solution.

ABSTRACT

The present invention relates to a method of isolating fully thioated single stranded antisense oligonucleotides from a biological solution, which method comprises the steps of contacting the biological solution with an immobilised metal ion adsorption chromatography (IMAC) resin to adsorb the antisense oligonucleotides to said resin and subsequently contacting the resin with an eluent under conditions that provide desorption of the antisense oligonucleotides from said resin. The invention also relates to the use of an immobilised metal ion adsorption chromatography (IMAC) resin for isolation of fully thioated single stranded antisense oligonucleotides from a biological solution.

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Fig 1

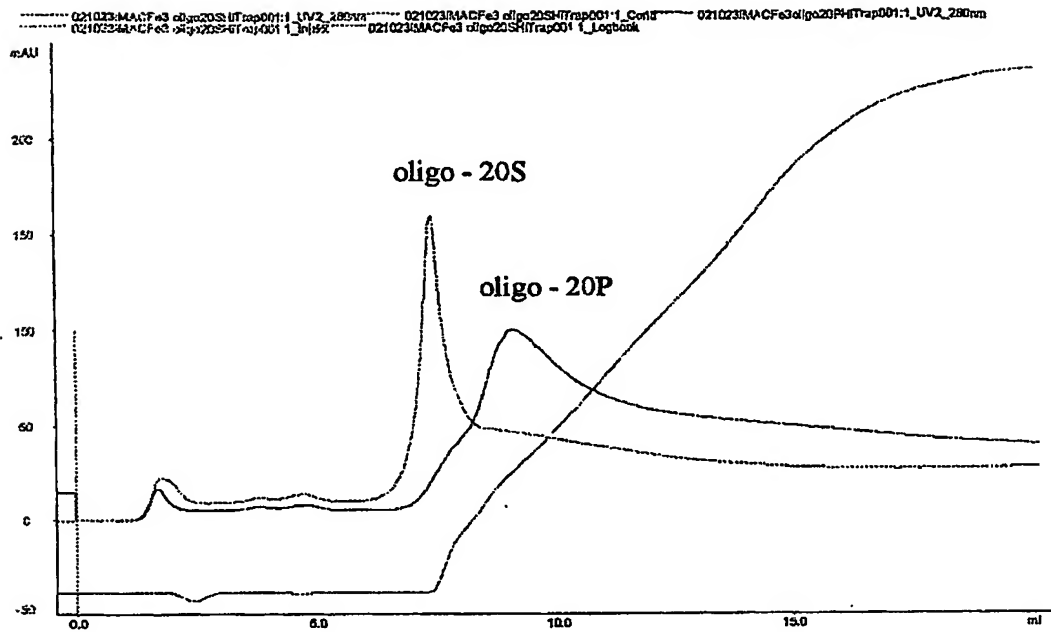


Fig 2

